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Evaluation of phenolic content, antioxidant capacity, and α -amylase inhibitory activity of clove, cinnamon, bay leaves, curry leaves, ginger, and fenugreek

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Abstract

Spices are widely recognized for their culinary significance and growing relevance as sources of bioactive compounds with potential health-promoting effects. The present study aimed to comparatively evaluate the functional properties of six commonly consumed culinary spices, clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), bay leaves (*Cinnamomum tamala*), curry leaves (*Murraya koenigii*), dried ginger (*Zingiber officinale*), and fenugreek (*Trigonella foenum-graecum*), with particular emphasis on phenolic content, antioxidant activity, and antidiabetic potential. Methanolic extracts of the spices were prepared and analyzed for total phenolic content using the Folin-Ciocalteu method, antioxidant activity using the DPPH free radical scavenging assay, and α -amylase inhibitory activity as an indicator of antidiabetic potential. The results revealed significant variation ($p < 0.05$) among the spices for all functional parameters evaluated. Clove exhibited the highest total phenolic content (269.96 mM GAE/g), followed by cinnamon and bay leaves, which were statistically at par. Correspondingly, clove demonstrated the highest DPPH radical scavenging activity (74.99%), with cinnamon and bay leaves showing comparable antioxidant capacity. In contrast, dried ginger exhibited the highest α -amylase inhibitory activity (52.78%), followed by clove, while cinnamon, curry leaves, and fenugreek showed comparatively lower enzyme inhibition. These findings indicate that antioxidant activity was largely associated with phenolic content, whereas α -amylase inhibition depended on specific bioactive constituents rather than total phenolic content alone. This suggests that spices such as clove, cinnamon, bay leaves, and dried ginger demonstrated considerable potential for incorporation into functional and health-oriented food products, particularly in formulations aimed at enhancing antioxidant capacity and glycemic regulation.

Keywords: Culinary spices, total phenolic content, antioxidant activity, DPPH radical scavenging, α -amylase inhibition, antidiabetic potential, bioactive compounds, functional foods

Introduction

Spices have formed an integral part of human diets and traditional medicine systems for millennia, valued not only for their distinctive aroma, taste, and color but also for their preservative and therapeutic attributes (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025; E. Sulieman *et al.*, 2023) [2, 1, 7]. Historically, spices were among the earliest natural additives used to enhance palatability, extend shelf life, and improve the safety of foods, particularly in tropical regions where ambient conditions favored rapid spoilage (Al-Habsi *et al.*, 2025; Zhang *et al.*, 2024; E. Sulieman *et al.*, 2023) [2, 22, 7]. Beyond their culinary role, spices have long been employed in traditional healing practices such as Ayurveda, Traditional Chinese Medicine, and Unani medicine, where they are prescribed for managing digestive disorders, metabolic ailments, inflammation, and infections (Aggarwal *et al.*, 2025; Rao & Gan, 2014; Hamidpour *et al.*, 2015) [1, 18, 10]. This longstanding ethnopharmacological use has prompted modern scientific interest in understanding the functional properties of spices (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025) [2, 1].

In recent decades, global dietary patterns have shifted markedly due to urbanization, sedentary lifestyles, and increased consumption of energy-dense, highly processed foods (Clemente-Suárez *et al.*, 2023) [6]. These changes have contributed to a sharp rise in lifestyle-related disorders such as obesity, type-2 diabetes mellitus, cardiovascular diseases, and

chronic inflammatory conditions (Clemente-Suárez *et al.*, 2023; Martemucci *et al.*, 2024) [6, 14]. Oxidative stress and postprandial hyperglycemia are recognized as key underlying mechanisms in the development and progression of several of these disorders (Martemucci *et al.*, 2024) [14]. Consequently, there is growing emphasis on dietary strategies that incorporate natural bioactive compounds capable of modulating oxidative stress, regulating carbohydrate metabolism, and improving overall metabolic health (Martemucci *et al.*, 2024; Parveen *et al.*, 2025) [14, 17]. Within this context, spices have gained renewed attention as affordable, culturally accepted, and food-grade sources of functional phytochemicals (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025) [2, 1].

Spices are rich reservoirs of diverse bioactive constituents, including polyphenols, flavonoids, phenolic acids, tannins, alkaloids, terpenoids, and essential oils (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025; Parveen *et al.*, 2025) [2, 1, 17]. These compounds exhibit a wide spectrum of biological activities, such as free radical scavenging, metal chelation, enzyme inhibition, modulation of inflammatory pathways, and antimicrobial action (Al-Habsi *et al.*, 2025; E. Sulieman *et al.*, 2023; Parveen *et al.*, 2025) [2, 7, 17]. Among these, phenolic compounds are of particular interest due to their strong antioxidant potential and their ability to interact with key metabolic enzymes (Parveen *et al.*, 2025) [17]. The antioxidant activity of spices plays a crucial role in neutralizing Reactive Oxygen Species (ROS), thereby preventing oxidative damage to lipids, proteins, and nucleic acids (Martemucci *et al.*, 2024; Parveen *et al.*, 2025) [14, 17]. This property is especially relevant in food systems, where oxidation is a major cause of quality deterioration, as well as in human physiology, where oxidative stress is implicated in aging and chronic disease development (Martemucci *et al.*, 2024; Parveen *et al.*, 2025) [14, 17].

In addition to antioxidant activity, the antidiabetic potential of spices has emerged as a significant area of research (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11]. One of the primary nutritional strategies for managing type-2 diabetes involves controlling postprandial blood glucose levels by slowing carbohydrate digestion and glucose absorption (Kashtoh & Baek, 2022) [13]. Enzymes such as α -amylase play a critical role in the hydrolysis of dietary starch into absorbable sugars (Kashtoh & Baek, 2022) [13]. Inhibition of α -amylase activity can therefore attenuate the rapid release of glucose into the bloodstream, reducing glycemic spikes after meals (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11]. Several plant-derived compounds, particularly phenolics and flavonoids, have been reported to exhibit α -amylase inhibitory activity (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11]. Spices, owing to their complex phytochemical composition, represent promising natural sources of such enzyme inhibitors, offering potential dietary support for glycemic management (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11].

Compared to synthetic antioxidants and antidiabetic agents, spices offer several advantages (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025; Parveen *et al.*, 2025) [2, 1, 17]. Synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT), though effective, have faced increasing scrutiny due to safety concerns and negative consumer perception (Parveen *et al.*, 2025) [17]. Similarly, synthetic enzyme inhibitors used in

diabetes management may be associated with gastrointestinal side effects and long-term compliance issues (Kashtoh & Baek, 2022) [13]. In contrast, spices are generally recognized as safe (GRAS), culturally familiar, and compatible with clean-label product development (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025) [2, 1]. Their multifunctional nature allows them to simultaneously impart sensory appeal and functional benefits, making them particularly attractive for incorporation into functional and value-added dairy and food products (Al-Habsi *et al.*, 2025; El-Sayed & Youssef, 2019) [2, 8].

The biological activity of spices depends on multiple factors, including botanical species, cultivar, geographical origin, maturity at harvest, processing conditions, and extraction methodology (Al-Habsi *et al.*, 2025; Zhang *et al.*, 2024; Aggarwal *et al.*, 2025) [2, 22, 1]. Furthermore, different spices exhibit distinct functional profiles; a spice rich in antioxidants may not necessarily show strong antidiabetic activity, and vice versa (Kashtoh & Baek, 2022) [13]. Therefore, systematic and comparative evaluation of spices using standardized analytical methods is essential to accurately characterize their functional potential and to guide their targeted application in food systems (Al-Habsi *et al.*, 2025; Zhang *et al.*, 2024) [2, 22].

Among commonly used culinary spices, clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), bay leaves (*Cinnamomum tamala*), curry leaves (*Murraya koenigii*), dried ginger (*Zingiber officinale*), and fenugreek (*Trigonella foenum-graecum*) hold particular relevance due to their widespread consumption and documented health-promoting properties (Aggarwal *et al.*, 2025; Pandey *et al.*, 2022; Rao & Gan, 2014; Batool *et al.*, 2019; Balakrishnan *et al.*, 2020; Mustafa & Chin, 2023; Ayustaningwarno *et al.*, 2024; Wani & Kumar, 2018; Faisal *et al.*, 2024) [1, 16, 18, 5, 4, 15, 3, 21, 9]. Clove is recognized for its high phenolic content, primarily attributed to eugenol, and is often recognized as one of the most potent natural antioxidants among spices (Pandey *et al.*, 2022; Hussain *et al.*, 2017) [16, 12]. Cinnamon is valued for its cinnamaldehyde-rich profile and has been associated with antioxidant, antimicrobial, and glucose-regulating effects (Rao & Gan, 2014; Hamidpour *et al.*, 2015; Sharifi-Rad *et al.*, 2021) [18, 10, 19]. Bay leaves, commonly used in South Asian cuisine, contain a variety of polyphenolic compounds and have been traditionally used for digestive and metabolic benefits (Batool *et al.*, 2019; Shehata *et al.*, 2025) [5, 20].

Curry leaves are a staple ingredient in Indian cooking and are reported to possess antioxidant, antidiabetic, and lipid-lowering properties, largely due to their carbazole alkaloids and phenolic constituents (Balakrishnan *et al.*, 2020) [4]. Dried ginger is widely recognized for its bioactive compounds such as gingerols and shogaols, which exhibit antioxidant, anti-inflammatory, and enzyme inhibitory activities (Mustafa & Chin, 2023; Ayustaningwarno *et al.*, 2024) [15, 3]. Fenugreek seeds, rich in soluble fiber, saponins, and phenolic compounds, have been extensively studied for their hypoglycemic and hypocholesterolemic effects (Wani & Kumar, 2018; Faisal *et al.*, 2024) [21, 9]. Although these spices are individually acknowledged for their health benefits, comparative evaluation under identical experimental conditions is limited, particularly with respect to simultaneous assessment of antioxidant and α -amylase inhibitory activities (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11].

In the context of functional food development, particularly in dairy-based foods, such comparative evaluation becomes even more critical (El-Sayed & Youssef, 2019) ^[8]. Dairy products are increasingly being reformulated to address consumer demand for reduced calorie, low-fat, and health-oriented alternatives (El-Sayed & Youssef, 2019) ^[8]. However, reformulation often influences sensory quality and oxidative stability (El-Sayed & Youssef, 2019) ^[8]. Spices, due to their dual sensory and functional roles, offer a promising strategy to overcome these challenges (Al-Habsi *et al.*, 2025; El-Sayed & Youssef, 2019) ^[2, 8]. Prior to their application in complex food matrices, it is essential to establish a clear functional profile of selected spices to ensure efficacy, compatibility, and consumer acceptance (Al-Habsi *et al.*, 2025; El-Sayed & Youssef, 2019) ^[2, 8].

Therefore, the present study was undertaken to systematically evaluate the functional properties of six commonly consumed spices, clove (Cl), cinnamon (Ci), bay leaves (BL), curry leaves (CL), dried ginger (DG), and fenugreek (FG), using standardized *in vitro* assays (Al-Habsi *et al.*, 2025; Kashtoh & Baek, 2022) ^[2, 13]. The study focused on determining total phenolic content as an indicator of bioactive content, antioxidant activity using the DPPH radical scavenging assay, and antidiabetic potential through α -amylase inhibition (Parveen *et al.*, 2025; Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) ^[17, 13, 11]. By generating comparative functional data under uniform experimental conditions, this investigation aims to provide a scientific basis for the rational selection of spices for functional food formulation (Al-Habsi *et al.*, 2025; El-Sayed & Youssef, 2019) ^[2, 8]. The findings are expected to contribute to the growing body of knowledge on spice-derived bioactives and support their targeted application in health-oriented food products (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025) ^[2, 1].

Materials and Methods

Selection, authentication, and preparation of spices

Six commonly consumed culinary spices, clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), bay leaves (*Cinnamomum tamala*), curry leaves (*Murraya koenigii*), dried ginger (*Zingiber officinale*), and fenugreek (*Trigonella foenum-graecum*), were selected based on their widespread dietary use and documented functional relevance (Aggarwal *et al.*, 2025; Al-Habsi *et al.*, 2025) ^[1, 2]. Whole spice materials, including clove flower buds, cinnamon bark, bay leaves, curry leaves, dried ginger rhizomes, and fenugreek seeds, were procured from authenticated local suppliers to ensure food-grade quality and freshness.

Prior to analysis, all spices were manually sorted to remove extraneous matter and washed thoroughly with potable water. Moisture-rich materials (bay leaves and curry leaves) were shade-dried at ambient temperature until a constant weight was achieved, while dried spices (clove, cinnamon bark, ginger, and fenugreek seeds) were used as received. The dried samples were ground using a laboratory grinder and sieved through a 20-30 mesh sieve to obtain uniform particle size, minimizing variability in extraction efficiency (Zhang *et al.*, 2024) ^[22]. Prepared spice powders were stored in airtight containers at room temperature, protected from light and moisture, until extraction.

Extraction procedure of spices for functional property analysis

Extraction of bioactive compounds from the selected spices was carried out following the method reported by Zhang *et al.* (2024) ^[22], with minor modifications to improve

extraction efficiency. Whole spice samples, including clove buds, cinnamon bark, bay leaves, curry leaves, dried ginger rhizomes, and fenugreek seeds, were ground into a fine powder using a laboratory grinder and sieved through a 20-30 mesh to obtain uniform particle size. The powdered samples were soaked in 95% methanol at a solid-to-solvent ratio of 1:5 (w/v) and allowed to macerate at room temperature for 24 h with intermittent shaking to facilitate the release of phenolic and other bioactive compounds. After maceration, the mixtures were subjected to ultrasonication at a frequency of 20 kHz for 10 min at room temperature to enhance extraction through cell wall disruption, as recommended for spice matrices (Zhang *et al.*, 2024; Parveen *et al.*, 2025) ^[22, 17]. The extracts were then filtered using Whatman No. 4 filter paper and centrifuged at 3000 rpm using a Remi centrifuge to remove insoluble residues. The clear supernatants were collected and stored at 4 °C until further analysis of functional properties, including total phenolic content, antioxidant activity, and α -amylase inhibitory activity (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025) ^[2, 1].

Determination of Total Phenolic Content (TPC)

Total phenolic content of the methanolic spice extracts was determined using the Folin-Ciocalteu colorimetric method, following the procedure described by Gupta (2013), which is widely applied for evaluating phenolic richness in spices and plant-derived foods (Al-Habsi *et al.*, 2025; Parveen *et al.*, 2025) ^[2, 17]. Briefly, aliquots of spice extracts were reacted with diluted Folin-Ciocalteu reagent, followed by neutralization with sodium carbonate solution. The reaction mixtures were allowed to stand at room temperature in the dark for color development, and absorbance was measured spectrophotometrically at 765 nm against a reagent blank. Gallic acid was used to construct the standard calibration curve, and total phenolic content was expressed as millimoles of gallic acid equivalents per gram of extract. All measurements were performed in triplicate to ensure analytical precision.

Determination of antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of spice extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and commonly employed for evaluating antioxidant capacity of spices and herbs (Parveen *et al.*, 2025; Al-Habsi *et al.*, 2025) ^[17, 2]. Methanolic DPPH solution was mixed with appropriately diluted spice extracts and incubated in the dark at room temperature to allow interaction between antioxidants and free radicals. The reduction in DPPH radical was monitored by measuring absorbance at 517 nm using a UV-visible spectrophotometer. Butylated hydroxyanisole (BHA) was used as the reference antioxidant standard. Antioxidant activity was expressed as percentage free radical scavenging activity relative to the control. All analyses were carried out in triplicate.

α -amylase inhibition assay

The antidiabetic potential of spice extracts was assessed by determining their inhibitory effect on pancreatic α -amylase activity using the dinitrosalicylic acid (DNS) method, following the protocol described by Hemlata *et al.* (2019) ^[11]. Methanolic spice extracts were incubated with α -

amylase enzyme in phosphate buffer (pH 6.9) under controlled conditions, followed by the addition of soluble potato starch as the substrate. After incubation, the enzymatic reaction was terminated using DNS reagent and heated to develop a colored complex corresponding to the amount of reducing sugars released. Absorbance was measured at 550 nm, and α -amylase inhibition was calculated relative to the control. This assay is widely used to evaluate the potential of plant-derived phytochemicals to modulate carbohydrate digestion and postprandial glycemic response (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11].

2.6 Statistical analysis

All experimental determinations were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation. Statistical analysis was carried out using one-way analysis of variance (ANOVA) to assess significant differences among the functional properties of the spice extracts. The statistical computations were performed using SPSS statistical software (IBM SPSS Statistics, Version 26.0, IBM Corp., Armonk, NY, USA). Differences among means were considered statistically significant at a probability level of $p < 0.05$.

Results and Discussions

Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) of methanolic extracts of the selected spices varied significantly, as determined by the Folin-Ciocalteu assay (Table 1; Figure 1). Phenolic content was quantified using a gallic acid calibration curve and expressed as millimoles of gallic acid equivalents per gram of extract (mM GAE/g). The observed variation in TPC emphasizes the intrinsic differences in phytochemical composition among spices, which are influenced by botanical origin, plant part used, and processing conditions (Al-Habsi *et al.*, 2025; Aggarwal *et al.*, 2025) [2, 1].

Among the six spices evaluated, clove exhibited the highest TPC (269.96 ± 19.98 mM GAE/g), followed closely by cinnamon (257.56 ± 4.10 mM GAE/g) and bay leaves (250.89 ± 10.05 mM GAE/g). Statistical analysis indicated that the TPC values of clove, cinnamon, and bay leaves were not significantly different from one another ($p > 0.05$), suggesting a comparably high phenolic richness among these spices. The elevated phenolic content of clove is primarily attributed to its high concentration of eugenol and related phenolic compounds, which are widely recognized for strong antioxidant properties (Pandey *et al.*, 2022; Hussain *et al.*, 2017) [16, 12]. Similarly, cinnamon is known to

be rich in cinnamaldehyde and other polyphenolic constituents, which contribute substantially to its antioxidant and metabolic regulatory functions (Rao & Gan, 2014; Hamidpour *et al.*, 2015; Sharifi-Rad *et al.*, 2021) [18, 10, 19]. Bay leaves have also been reported to contain a diverse array of phenolic acids and flavonoids, supporting their traditional use for digestive and metabolic health benefits (Batool *et al.*, 2019; Shehata *et al.*, 2025) [5, 20].

In contrast, significantly lower TPC values ($p < 0.05$) were observed for curry leaves, dried ginger, and fenugreek. Curry leaves exhibited a moderate phenolic content (191.63 ± 5.78 mM GAE/g), while dried ginger (90.33 ± 3.53 mM GAE/g) and fenugreek (89.96 ± 2.28 mM GAE/g) showed comparatively lower phenolic levels. Although curry leaves are recognized for their bioactive carbazole alkaloids and antioxidant potential, their phenolic concentration was markedly lower than that of clove and cinnamon under the extraction conditions employed in the present study (Balakrishnan *et al.*, 2020) [4]. The relatively low TPC observed for dried ginger is consistent with earlier reports indicating that ginger's bioactivity is driven more by pungent phenolics such as gingerols and shogaols rather than total phenolic abundance (Mustafa & Chin, 2023; Ayustaningwarno *et al.*, 2024) [15, 3]. Similarly, fenugreek seeds, despite their well-documented hypoglycemic and hypocholesterolemic effects, are known to exert biological activity largely through soluble fiber, saponins, and steroidal compounds rather than high phenolic concentration alone (Wani & Kumar, 2018; Faisal *et al.*, 2024) [21, 9].

Overall, the order of total phenolic content among the evaluated spices was as follows: clove > cinnamon > bay leaves > curry leaves > dried ginger $\approx$ fenugreek. This trend aligns well with previous investigations reporting clove as the richest source of phenolic compounds among commonly consumed spices (Pandey *et al.*, 2022; Al-Habsi *et al.*, 2025) [16, 2].

The significant differences in TPC observed in this investigation underscore the importance of comparative evaluation of spices under standardized analytical conditions. High phenolic content is often associated with enhanced antioxidant potential and improved oxidative stability in food systems, particularly in functional and dairy-based formulations (Al-Habsi *et al.*, 2025; El-Sayed & Youssef, 2019) [2, 8]. Therefore, spices such as clove, cinnamon, and bay leaves, which demonstrated superior phenolic richness, may be considered promising candidates for incorporation into functional food products aimed at enhancing antioxidant capacity.

Table 1: Functional properties of selected spices

Name of spices	TPC % Concentration (mM GA (eq.)/g)	Free radical scavenging activity (% DPPH scavenging)	Anti-Diabetic activity (% amylase inhibition)
Clove (Cl)	$269.96^a \pm 19.98$	$74.99^a \pm 0.61$	$47.22^a \pm 3.67$
Cinnamon (Ci)	$257.56^a \pm 4.1$	$68.74^b \pm 1.35$	$31.94^c \pm 3.67$
Bay leaves (BL)	$250.89^a \pm 10.05$	$71.60^{a,b} \pm 1.31$	$43.06^{a,b} \pm 3.67$
Curry leaves (CL)	$191.63^b \pm 5.78$	$59.08^c \pm 0.55$	$29.17^c \pm 2.41$
Dried ginger (DG)	$90.33^c \pm 3.53$	$16.22^d \pm 2.24$	$52.78^d \pm 3.67$
Fenugreek (FG)	$89.96^c \pm 2.28$	$20.41^d \pm 2.44$	$33.33^{b,c} \pm 2.41$
SEm	9.729	1.59	3.306
CD (0.05)	29.980	4.91	10.188
CV%	8.789	5.32	14.467

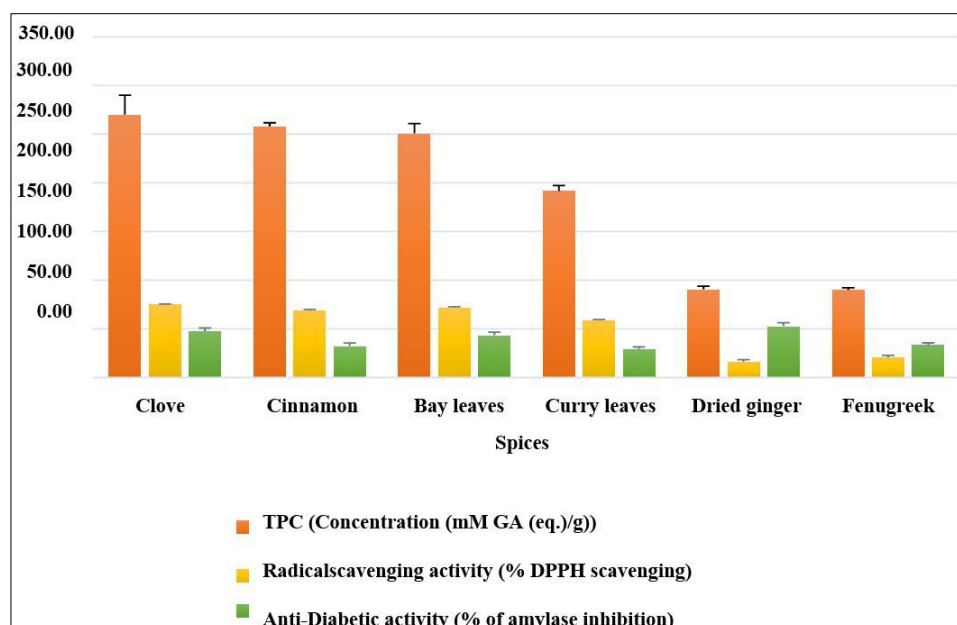


Fig 1: Functional properties of selected spices

Free Radical Scavenging Activity (DPPH assay)

Antioxidant activity of the selected spice extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, a widely accepted and reliable method for assessing the hydrogen-donating ability of plant-derived antioxidants (Parveen *et al.*, 2025; Al-Habsi *et al.*, 2025) [17, 2]. The DPPH radical is a stable nitrogen-centered free radical that exhibits a deep violet color in methanolic solution. Upon interaction with antioxidant compounds capable of donating hydrogen atoms or electrons, DPPH is reduced to a non-radical form, resulting in a measurable decrease in absorbance and a visible color change from purple to pale yellow. This reaction provides a rapid and reproducible estimate of the antiradical capacity of spice extracts and has been extensively applied for screening antioxidant potential in spices and herbs (Aggarwal *et al.*, 2025; Parveen *et al.*, 2025) [1, 17].

In the present study, significant variation in DPPH radical scavenging activity was observed among the evaluated spices (Table 1; Figure 1). Clove exhibited the highest free radical scavenging activity (74.99%), followed by bay leaves (71.60%) and cinnamon (68.74%). Statistical analysis revealed that the scavenging activity of clove was not significantly different ($p > 0.05$) from that of cinnamon and bay leaves, indicating comparable antioxidant efficacy among these three spices. The superior DPPH scavenging capacity of clove can be attributed primarily to its exceptionally high phenolic content, particularly eugenol and related phenolic compounds, which possess strong hydrogen-donating ability and resonance-stabilized radical structures (Pandey *et al.*, 2022; Hussain *et al.*, 2017) [16, 12]. Similarly, the antioxidant activity of cinnamon is largely associated with cinnamaldehyde, procyanidins, and other polyphenols that effectively neutralize free radicals (Rao & Gan, 2014; Hamidpour *et al.*, 2015; Sharifi-Rad *et al.*, 2021) [18, 10, 19].

Bay leaves also demonstrated strong radical scavenging activity, consistent with their reported richness in polyphenolic acids and flavonoids. Previous studies have highlighted the antioxidant and metabolic health benefits of bay leaves, linking these effects to their phenolic composition and essential oil constituents (Batool *et al.*,

2019; Shehata *et al.*, 2025) [5, 20]. The close correspondence between total phenolic content and DPPH scavenging activity observed for clove, cinnamon, and bay leaves suggests a positive association between phenolic concentration and antioxidant potential, a relationship widely documented in spice-based antioxidant studies (Al-Habsi *et al.*, 2025; Parveen *et al.*, 2025) [2, 17].

In contrast, curry leaves, dried ginger, and fenugreek exhibited significantly lower ($p < 0.05$) DPPH radical scavenging activity, with values of 59.08%, 16.22%, and 20.41%, respectively. Although curry leaves are known to possess antioxidant activity, their comparatively lower scavenging capacity may be attributed to a reduced concentration of extractable phenolics under the methanolic extraction conditions used in this study. Curry leaves are reported to derive much of their bioactivity from carbazole alkaloids and other non-phenolic compounds, which may not exhibit strong hydrogen-donating ability in the DPPH system (Balakrishnan *et al.*, 2020) [4].

Dried ginger showed markedly low DPPH scavenging activity despite its well-documented antioxidant and anti-inflammatory properties. This apparent discrepancy can be explained by the fact that ginger's biological activity is largely governed by pungent compounds such as gingerols and shogaols, which may exert antioxidant effects through mechanisms other than direct free radical scavenging measured by the DPPH assay (Mustafa & Chin, 2023; Ayustaningwarno *et al.*, 2024) [15, 3]. Similarly, fenugreek exhibited relatively low radical scavenging activity, which aligns with reports suggesting that fenugreek's health benefits are primarily mediated through soluble fiber, saponins, and steroidal compounds rather than high levels of phenolic antioxidants (Wani & Kumar, 2018; Faisal *et al.*, 2024) [21, 9].

Overall, the order of DPPH radical scavenging activity among the spices evaluated was: clove > bay leaves > cinnamon > curry leaves > fenugreek > dried ginger. This trend is in agreement with previous studies reporting superior antioxidant activity of clove and cinnamon compared to other commonly used spices (Pandey *et al.*, 2022; Al-Habsi *et al.*, 2025) [16, 2]. The findings further underscore that antioxidant potential among spices is highly

dependent on phytochemical composition and that high total phenolic content generally, but not universally, correlates with strong radical scavenging activity.

From a functional food development perspective, spices demonstrating high DPPH scavenging activity, such as clove, cinnamon, and bay leaves, may be particularly valuable for enhancing oxidative stability and health functionality in food systems, including dairy-based formulations (El-Sayed & Youssef, 2019; Al-Habsi *et al.*, 2025) [8, 2].

Anti-diabetic activity (% α -amylase inhibition)

α -Amylase is a key digestive enzyme responsible for the hydrolysis of dietary starch into oligosaccharides and simple sugars, which are subsequently absorbed as glucose. Inhibition of α -amylase activity is a well-established nutritional strategy for controlling postprandial hyperglycemia and is therefore considered a relevant target in the dietary management of type-2 diabetes mellitus and obesity (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11]. Natural α -amylase inhibitors derived from food-grade plant sources are particularly attractive because they offer glycemic regulation with reduced risk of adverse gastrointestinal effects commonly associated with synthetic antidiabetic drugs (Kashtoh & Baek, 2022; Aggarwal *et al.*, 2025) [13, 1]. In this context, the present study evaluated the α -amylase inhibitory potential of six commonly consumed dietary spices, clove (Cl), cinnamon (Ci), bay leaves (BL), dried ginger (DG), curry leaves (CL), and fenugreek (FG), using an *in vitro* enzyme inhibition assay. The results are presented in Table 1 and Figure 1.

The findings revealed marked variation in α -amylase inhibitory activity among the evaluated spices. Dried ginger exhibited the highest percentage inhibition of α -amylase (52.78%), followed by clove (47.22%). Statistical analysis indicated that the inhibitory activities of dried ginger and clove were not significantly different from each other ($p > 0.05$), suggesting comparable antidiabetic potential for these two spices under the experimental conditions employed. The strong α -amylase inhibition observed for dried ginger may be attributed to its bioactive pungent compounds, particularly gingerols and shogaols, which have been reported to interact with carbohydrate-digesting enzymes and modulate glucose metabolism through enzyme inhibition and insulin-sensitizing mechanisms (Mustafa & Chin, 2023; Ayustaningwarno *et al.*, 2024) [15, 3]. These compounds are known to exert biological effects that are not solely dependent on total phenolic concentration, explaining the high enzyme inhibition despite relatively low phenolic content and antioxidant activity.

Clove also demonstrated pronounced α -amylase inhibitory activity, consistent with previous reports highlighting the role of eugenol and related phenolic constituents in enzyme inhibition and glycemic control (Pandey *et al.*, 2022; Hussain *et al.*, 2017) [16, 12]. The ability of clove extract to inhibit α -amylase may be linked to synergistic interactions between phenolics and essential oil components, which collectively interfere with enzyme-substrate binding (Kashtoh & Baek, 2022) [13]. This dual antioxidant and enzyme-inhibitory functionality underscores the relevance of clove as a multifunctional spice for metabolic health.

Bay leaves exhibited moderate α -amylase inhibition (43.06%), followed by fenugreek (33.33%) and curry leaves (29.17%), while cinnamon showed the lowest inhibitory

activity (31.94%) among the spices tested. The intermediate inhibition observed for bay leaves aligns with previous studies reporting their potential to modulate carbohydrate metabolism through polyphenolic compounds and essential oils (Batool *et al.*, 2019; Shehata *et al.*, 2025) [5, 20]. Fenugreek, despite its traditional reputation for antidiabetic effects, showed comparatively lower enzyme inhibition in the present *in vitro* assay. This observation is consistent with literature indicating that fenugreek's hypoglycemic effects are primarily mediated through soluble dietary fiber, delayed gastric emptying, and insulin sensitization rather than direct inhibition of digestive enzymes (Wani & Kumar, 2018; Faisal *et al.*, 2024) [21, 9].

Similarly, curry leaves exhibited relatively low α -amylase inhibition, which may be attributed to their bioactivity being driven largely by carbazole alkaloids and lipid-modulating compounds rather than strong carbohydrate-digesting enzyme inhibitors (Balakrishnan *et al.*, 2020) [4]. Cinnamon also demonstrated lower inhibitory activity despite its high phenolic content and antioxidant capacity. This finding highlights that antioxidant potential does not necessarily correlate with α -amylase inhibitory activity, as enzyme inhibition depends on specific molecular interactions rather than total phenolic abundance alone (Kashtoh & Baek, 2022; Hemlata *et al.*, 2019) [13, 11].

Overall, the order of α -amylase inhibitory activity among the evaluated spices was as follows: dried ginger > clove > bay leaves > fenugreek > curry leaves > cinnamon. These results emphasize the functional heterogeneity of spices and demonstrate that different spices exert metabolic benefits through distinct biochemical mechanisms. While spices such as clove and cinnamon excel in antioxidant activity, dried ginger exhibits superior antidiabetic potential through effective α -amylase inhibition.

From a functional food development perspective, particularly in the formulation of reduced-calorie and diabetic-friendly dairy products, spices such as dried ginger and clove may offer significant advantages by contributing to glycemic control without compromising sensory quality (El-Sayed & Youssef, 2019; Al-Habsi *et al.*, 2025) [8, 2].

Conclusion

The present study evaluated the functional properties of six commonly consumed culinary spices, clove, cinnamon, bay leaves, curry leaves, dried ginger, and fenugreek, by assessing their total phenolic content, antioxidant activity, and α -amylase inhibitory potential using standardized *in vitro* assays. The findings demonstrated marked variability among spices in their functional profiles, underscoring the importance of comparative evaluation when selecting spices for targeted health and food applications. Clove, cinnamon, and bay leaves exhibited significantly higher total phenolic content and superior DPPH radical scavenging activity, indicating strong antioxidant potential that can be attributed to their rich phenolic composition. In contrast, dried ginger showed the highest α -amylase inhibitory activity, highlighting its pronounced antidiabetic potential despite relatively lower phenolic content and antioxidant capacity. This observation confirms that enzyme inhibition and glycemic modulation depend on specific bioactive constituents rather than total phenolic concentration alone. Curry leaves and fenugreek demonstrated moderate functional activity, suggesting their bioactivity may be mediated through alternative mechanisms such as alkaloids,

dietary fiber, and saponins. Overall, the study reveals that culinary spices possess distinct and complementary functional attributes, with some excelling in antioxidant activity and others in antidiabetic potential. These results emphasize that a multi-parameter evaluation approach is essential for accurate functional characterization of spices. From an application perspective, spices such as clove, cinnamon, bay leaves, and dried ginger show considerable promise for incorporation into functional and health-oriented food products, particularly in formulations aimed at enhancing oxidative stability and glycemic regulation. Further investigations focusing on bioavailability, synergistic interactions, and validation in food matrices and *in vivo* systems are warranted to fully exploit the functional potential of these spices in dietary interventions.

References

- Aggarwal D, Sachdev B, Agarwal A, Nigam S, Raizada P, Siddhu A. Traditional herbs and spices: bioactive properties and potential food applications. *Discover Food*. 2025;5(1):245. DOI:10.1007/s44187-025-00546-9.
- Al-Habsi N, Al-Khalili M, Haque SA, Al Akhzami N, Gonzalez-Gonzalez CR, Al Harthi S, *et al.* Herbs and spices as functional food ingredients: A comprehensive review of their therapeutic properties, antioxidant and antimicrobial activities, and applications in food preservation. *J Funct Foods*. 2025;129:106882. DOI:10.1016/j.jff.2025.106882.
- Ayustaningwarno F, Anjani G, Ayu AM, Fogliano V. A critical review of ginger's (*Zingiber officinale*) antioxidant, anti-inflammatory, and immunomodulatory activities. *Front Nutr*. 2024;11:1364836. DOI:10.3389/fnut.2024.1364836.
- Balakrishnan R, Vijayraja D, Jo H, Ganesan P, Su-Kim I, Choi K. Medicinal profile, phytochemistry, and pharmacological activities of *Murraya koenigii* and its primary bioactive compounds. *Antioxidants*. 2020;9(2):101. DOI:10.3390/antiox9020101.
- Batool S, Khera RA, Hanif MA, Ayub MA. Bay leaf. In: *Medicinal Plants of South Asia*. c2019. p. 63. DOI:10.1016/B978-0-08-102659-5.00005-7.
- Clemente-Suárez VJ, Beltrán-Velasco AI, Redondo-Flórez L, Martín-Rodríguez A, Tornero-Aguilera JF. Global impacts of western diet and its effects on metabolism and health: A narrative review. *Nutrients*. 2023;15(12):2749. DOI:10.3390/nu15122749.
- Suliman EAM, Abdallah EM, Alanazi NA, Ed-Dra A, Jamal A, Idriss H, *et al.* Spices as sustainable food preservatives: A comprehensive review of their antimicrobial potential. *Pharmaceuticals*. 2023;16(10):1451. DOI:10.3390/ph16101451.
- El-Sayed SM, Youssef AM. Potential application of herbs and spices and their effects in functional dairy products. *Heliyon*. 2019;5(6):e01989. DOI:10.1016/j.heliyon.2019.e01989.
- Faisal Z, Irfan R, Akram N, Irfan Manzoor HM, Aabdi MA, Anwar MJ, *et al.* The multifaceted potential of fenugreek seeds: From health benefits to food and nanotechnology applications. *Food Sci Nutr*. 2024;12(4):2294-2306. DOI:10.1002/fsn3.3959.
- Hamidpour R, Hamidpour M, Hamidpour S, Shahlari M. Cinnamon: From traditional applications to novel effects on angiogenesis inhibition in cancer cells and prevention of Alzheimer's disease. *J Tradit Complement Med*. 2015;5(2):66-70. DOI:10.1016/j.jtcme.2014.11.008.
- Hemlata B, Pornima G, Tukaram K, Pankaj B. *In vitro* anti-amylase activity of some Indian dietary spices. *J Appl Biol Biotechnol*. 2019;7(4):70-74.
- Hussain S, Rahman R, Mushtaq A, Zerey-Belaskri AE. Clove: A review of a precious species with multiple uses. *Int J Chem Biochem Sci*. 2017;11:129-133.
- Kashtoh H, Baek K. New insights into the latest advancement in α -amylase inhibitors of plant origin with anti-diabetic effects. *Plants*. 2022;12(16):2944. DOI:10.3390/plants12162944.
- Martemucci G, Khalil M, Di Luca A, Abdallah H, Gabriella A. Comprehensive strategies for metabolic syndrome: Nutrition, dietary polyphenols, physical activity, and lifestyle modifications. *Metabolites*. 2024;14(6):327. DOI:10.3390/metabo14060327.
- Mustafa I, Chin NL. Antioxidant properties of dried ginger (*Zingiber officinale* Roscoe) var. Bentong. *Foods*. 2023;12(1):178. DOI:10.3390/foods12010178.
- Pandey VK, Shams R, Singh R, Dar AH, Pandiselvam R, Rusu AV, *et al.* Clove (*Caryophyllus aromaticus* L.) essential oil and its significance in edible coatings for food applications. *Front Nutr*. 2022;9:987674. DOI:10.3389/fnut.2022.987674.
- Parveen B, Rajinikanth V, Narayanan M. Natural plant antioxidants for food preservation and emerging trends in nutraceutical applications. *Discover Appl Sci*. 2025;7(8):845. DOI:10.1007/s42452-025-07464-6.
- Rao PV, Gan SH. Cinnamon: A multifaceted medicinal plant. *Evid Based Complement Alternat Med*. 2014;2014:642942. DOI:10.1155/2014/642942.
- Sharifi-Rad J, Dey A, Koirala N, Shaheen S, Omari NE, Salehi B, *et al.* *Cinnamomum* species: Phytochemistry, pharmacology and toxicological safety. *Front Pharmacol*. 2021;12:600139. DOI:10.3389/fphar.2021.600139.
- Shehata AI, Taha SA, Elmaghraby AM, Elhetawy AI, Mohamed Srour T, El Basuni MF, *et al.* Effects of dietary bay leaf (*Laurus nobilis*) aqueous extract on growth performance and immunity in Nile tilapia. *Aquaculture*. 2025;599:742155. DOI:10.1016/j.aquaculture.2025.742155.
- Wani SA, Kumar P. Fenugreek: A review on its nutraceutical properties and utilization in food products. *J Saudi Soc Agric Sci*. 2018;17(2):97-106. DOI:10.1016/j.jssas.2016.01.007.
- Zhang D, Ahlivia EB, Bruce BB, Zou X, Battino M, Savić D, *et al.* Re-use of spice by-products: Bioactive compounds and significance in active packaging. *Foods*. 2024;14(14):2445. DOI:10.3390/foods14142445.